J. Glob. Innov. Agric. Sci.,2021, 9(1):29-33.
ISSN (Online):2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/9.932
http://www.jgiass.com

OVIPOSITION DETERRENT EFFECT OF FOUR PLANT EXTRACTS ON *Cydia pomonella* L. (LEP.: TORTRICIDAE)

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Cydia pomonella L. (Lepidoptera: Tortricidae) is one of the most important pests that causes economic loss in apple production. Chemical insecticides are used extensively to control *C. pomonella.* This study investigated the oviposition deterrent effect of extracts obtained from *Tagetes patula* L. (Asteraceae), *Tanacetum vulgare* L. (Asteracea), *Aleo vera* (L.) (Liliaceae) and *Hyoscyamus niger L. (Solanaceae)* on *C. pomonella* under laboratory conditions. For this purpose, green apple fruits of the similar size were selected for the experiment. Apples were sprayed with two concentrations (5 and 10%) of either one of the plants extracts with a small hand sprayer and placed in Petri dish (one apple per dish). Distilled water was used as control spray treatment. After the sprayed apples dried, each petri dish was placed in small cages in which two male and one female of *C. pomonella* were released for coupling for 24h. Daily monitoring was conducted for next fourteen days in controlled growth cabinet and the total number of eggs laid was recorded. The extract of *A. vera* exhibited the highest deterrence effect (89.92%) at maximum concentration (10%). The extracts of *H. niger* and *T. vulgare,* were 88.48% and 84.89% deterrent respectively with the same concentration. Whereas, deterrence effect of extracts from *T. patula,* was 69.06% at the same concentration. The percent oviposition deterrence increased with increased concentration of plant extract. The highest index of egg laying was with extract of *A. vera.*

Keywords: Botanicals**,** *Malus communis* L., insect pests, Codling moth, plant protection, oviposition, yield loss.

INTRODUCTION

Apple (*Malus communis* L.) is a species commonly grown in the world. Turkey ranks 4th in the world in terms of apple production (FAO, 2017). According to data of 2020, 3.878,550 tons of apples were produced in Turkey (TUIK, 2020). However, several pests limit production during apple growing period. The most important of these is Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) is a major threatening pest in Turkey as well as in the world. *C. pomonella* has caused significant economic losses in apple cultivation. Without any plant protection measures, damage may reach 60-95% of apple production (Kuyulu and Genc, 2018). This pest is also the main pest in pear, quince and walnut (Howell *et al*., 1992). Larvae feeds directly on fruit, causing serious economic losses. If the pest is not controlled, it results in 100% yield loss. Good practice, mechanical, biological, biotechnological and chemical control methods are applied in the control of the pest. However, chemical control is the most preferred method in Turkey as in the whole world due to its easy application and attainment of effective results in a short time. In order to prevent negative effects caused by the unconscious use of synthetic insecticides on humans and the ecosystem, efforts to research and apply alternative insecticide inputs are increasing. In recent years, significant number of relative studies using plant extracts in pest control in Turkey and elsewhere in the world (Doğan *et al.,* 2016., Ahmed *et al.,* 2019). Plants produce many compounds that negatively affect insects. These negative effects include insecticidal, repellency, anti-feeding, oviposition deterrence and growth retardants effects. The major advantage of using plants extracts is the target specific action on the insects without destroying the natural enemies along with provision of residue free food with safe environment.

Hyoscyamus niger (henbane, black henbane, stinking nightshade) belongs to the Solanaceae family is a poisonous plant. Its plant parts (seeds, leaves, roots, fruits) are rich in atropine, hyoscine and scopolamine. These parts were used as local anesthetic for treating pains in ancient times (Yucel and

Erdogan, P. and A. Ozdem. 2021. Oviposition deterrent effect of four plant extracts on *cydia pomonella* l. (*lep.: tortricidae*). J. Glob. Innov. Agric. Sci. 9:29-33.
[Received 20 Oct. 2020; Accepted 1 Jan- 2020; Published (online) 28 Mar. 2021]

Yilmaz, 2014). Extracts from *H. niger* flower were insecticidal effective *Anopheles* spp. mosquito larvae (Behravan, 2017). Akkol *et al*. (2020) stated that the larvae have small size, contracted and weak cuticle.

Tagetes patula (Asteraceae), French Marigolds are compact bushy annuals from Mexico. Flowers of this plant come in various color combinations like red, orange and yellow. Infusions taken from dried leaves or florets are used as medicine. The essential oil extracted from *T. patula* are highly effective and can be employed as a potential residual pesticide used against bedbugs (Politi *et al*., 2016), antifungal activity for treating candidiasis (Dutta) and other fungal infections (Mares, 2004; Romagnoli, 2005) in plants. Erdogan (2017) revealed that the marigold is attractive to two-point red spider individuals.

Tanacetum vulgare L. (common name Tansy; Asteracea), is a perennial herbaceous flowering plant, native of temperate regions of Asia and Europe. Later on, introduced to the rest of the world, including North America, and became invasive in some areas. Tansy is a cultivated plant used as insect repellent and in the worm winding type of embalming (*Mitich*, 1992; *Zimdahl*, 1989) and is effective for biological pest control as a companion plant in potato to repel the Colorado potato beetle and reduces the beetle population by 60 to 100% (Schearer, 2004).

Aloe vera (true aloe) is a widely known plant species that is cultivated for pharmaceutical purposes (*Le Cain and Sheley, 2002). A. vera* is used as folk or alternative medicine by humans and *Aloe* species are generally acknowledged as plant with cosmetic and medicinal properties (Fentaw *et al.* 2013).

The purpose of this study was to determine the oviposition deterrent effect of *T. vulgare, A. vera*, *T. patula* and *H. niger* extracts against Codling moth.

MATERIALS AND METHODS

***Rearing larvae and adults*:** Codling moth mass production was made on artificial diet according to Bathon (1991). Codling moth culture used in the experiment was established using larvae collected from apple orchards in Ankara province. These apples were placed in a cage (40 x 45x 50 cm) with a chiffon lid. Corrugated cardboard strips were placed to allow the larvae to pupate when mature. Pupae were gently harvested with soft forceps and transferred to screen cages (20 x 20 x 20 cm). After the adults emerged, they were placed in spawning cages. The culture of Codling moth was grown in controlled growth chambers with temperature (25±1°C), humidity (65±5%) and 16 hrs light photoperiod conditions (Vetter *et al.*, 1989).

***Plants material*:** The plants *T. vulgare and H. niger* were collected in and around Ankara province in 2017. Different plant parts (leaves, flowers, stems) were used to obtain the extract. *T. patula* was grown in the greenhouse. *A. vera* were grown in pots at room conditions.

Table 1. Plants used in ovposition effect on Codling moth

Scientific name	Family	Tissue used
Tagetes patula L.	Asteraceae	Flowers, leaves, buds
Tanacetum vulgare L.	Asteraceae	Flowers, leaves, buds
Hyoscyamus niger L.	*Solanaceae*	Flowers, leaves, buds
Aleo vera L.	Liliaceae	Buds

Preparing extracts: The plant material was cut at the same level and all plant material was used for extraction. The plant material was thereafter dried under laboratory conditions. Oven-dried plant material was ground to obtain fine powder. For extraction, 400 ml of ethanol (80%) was added to 200 g of dried powdered plant material and incubated for 72 hours. The mixture was placed in Soxhlet for 5-6 hours. The extract was filtered using Whatman No.1 filter paper in a Bucher funnel followed by concentrated under reduced pressure using a rotary evaporator (50-60°C). The crude extract was kept in glass bottles at 4°C (stock plant extract).

***The plant extracts oviposition deterrent effect on Codling moth*:** Individuals were distinguished into male or females during the pupal stage. Two males and one female were allowed for mating to live in a cage (15x15x10 cm), lined with tulle without food for 24h. After 24h, adults were subjected to cages supplemented with extract treated apples. These apples were sprayed with the prepared concentrations of the extracts with a small hand sprayer. The treated apples dried for half an hour were placed in petri dish (one per dish). Then each petri dish with an apple was placed in cages (30×30×30cm) with 1 female and 2 males. The trial was done in triplicate and pure water was used as control treatment spray. Distilled water (dH_2O) was used for making extracts concentrations and TritonX100 was used as the spreader adhesive. Two concentrations (5 and 10%) of each extract were sprayed. The experiment was designed as completely randomized design and counts were performed daily for 14 days. During daily controls, eggs on each apple were counted, recorded and removed. The experiments were conducted in controlled growth chambers with 25±1°C, 65±5% humidity and 16 hours light and 8 hours dark photoperiod conditions.

***Statistical analysis*:** The results obtained were subjected to a variance analysis using the SPSS program 20.6. The mean values were compared by using Duncan's test (P=0.05). The percent oviposition deterrent effect (PODE) was tabulated by using the methodology reported by Thaukar and Gupta (2013).

$$PODE = \frac{\text{Number of eggs laid in control- Number of eggs laid in treatment}}{\text{Number of eggs laid in treatment}} \times 100$$

Index of egg laying (IE) was tabulated by using method reported by Arivoli and Tennyson (2013).

$$IE = \frac{\text{Number of eggs laid in control- Number of eggs laid in treatment}}{\text{Number of eggs laid in control + Number of eggs laid in treatment}} \times 100$$

RESULTS

The highest number of eggs was recorded in control (Table 2). This was followed by the second concentration of *T. patula* extract. The least number of eggs were determined in apples treated with the highest concentration of *A. vera* extract. The lowest oviposition deterrence effect was determined by *T. patula* extract (5%). The PODE enhanced with elevated plant extracts concentration (Table 2). The highest index of egg laying was in group treated with extracts of *A. Vera* while the lowest index of egg laying was determined at the low concentration (5%) of *T. patula* extract. According to statistical analysis for oviposition deterrent effect, there was no difference among the high concentrations of extracts of *T. vulgare, A. vera* and *H. niger*. Other concentrations were in a different group (F=3.01; P>0.05). Also, in statistical analysis for index of egg laying, the high concentrations of extracts of *A. vera and H. niger* were taken the same group (Table 2). Other concentrations were in a different group (F=4.62; P>0.05).

Table 2. Oviposition deterrent effect of plant crude extracts against apple Codling moth.

Treatments	Concen-trations (%)	Number of eggs	Oviposition deterrent effect (%)	Index of egg laying (%)
H. niger	5	29	79.13±2.91ab*	65.47±4.84b
	10	16	88.48±2.06a	79.35±3.11a
A. vera	5	28	79.85±3.42ab	66.46±3.21b
	10	14	89.92±2.06a	81.69±2.46a
T. patula	5	61	56.11±3.65c	39.00±3.06d
	10	43	69.06±3.89b	52.74±3.56c
T. vulgare	5	31	77.69±3.42b	63.52±3.89b
	10	21	84.89±2.23a	73.75±2.89ab
	Control	139		

*Means within rows followed by the same uppercase letter are not significantly different (Duncun's multiple range test).

DISCUSSION

The PODE values of the extracts of *T. vulgare, H. niger, T. patula* and *A. vera* was investigated on Codling moth. All the extracts were found effective, especially, the extracts of *A. vera, H. niger and T. vulgare* exhibited strong oviposition deterrent effect on Codling moth. This study is the first ever record to determine the oviposition deterrent effect of extracts from T. *vulgare, H. niger, T. patula* and *A. vera* on Codling Moth, and so far, no studies have been conducted regarding the oviposition of these plants on Codling moth. However, there are studies evaluating the biological activities of different plant extracts and plant oils on Codling moth, for example, Cariac *et al.* (2003) used crude plant extracts of different plants like *Gossypium hirsutum* L., *Glycine max* (L.) Merr. and *Diplotaxis tennuifolia* (L.) De Candolle and reported drop in the fecundity of Codling moth. Likewise, Kovanci (2015) reported that the microencapsulated cardamom (MEC-C) and eucalyptol (MEC-E) showed significant oviposition deterrence activity against gravid females of Codling moth with MEC-C recording 84% effective repellency at 100 mg mL^{-1}. Suomi *et al*. (1986) identified oils from *Artemisia absinthium* L., *Allium sativum* L. and *Tanacetum vulgare* L. and showed that these had strong repellent effect on neonate codling moth larvae. Gökçe *et al*. (2018) reported high levels of both antioviposition and ovicidal activity against female Codling moth by using *Xanthium strumarium* and *H. lupulus* extracts. In addition, *D. tennuifolia* extracts were observed to cause a large reduction in codling moth fecundity (Agullo *et al.,* 1987). In another study, Hayes and Smith (1994) determined that extract of *G. hirsutum* caused a decrease in egg production of *C. pomonella*.

There is some literature about extract of *H. niger*. It was determined that plant extracts of *H. niger* had mortality effect of over 90% against *C. pipiens* (Yigit *et al.,* 2019). Akkol *et al*. (2020) determined that extract of *H. niger* had insecticidal effects on *Lucilia sericata* Meigen, (Diptera: Calliphoridae). The flower extract of *H. niger* was the most effective in destroying the *Anopheles* spp. mosquito larvae (Behravan *et al*., 2017). Moreover, Gamal (2012) revealed that henbane extracts had insecticidal effect on cabbage aphid. In another study, it was reported that the mortality of nymphs and adults of *Myzus persicae* Sulzer (Hemiptera: Aphididae) were 71.64 and 65.00%, respectively at 12% concentration extract of *H. niger* (Erdogan and Yildirim, 2013).

T. vulgare is a medicinal plant and many studies reflect the impact of *T. vulgare* extracts on other insects like *Pieris rapae* L. on treated cabbage leaf disks (Goldstain and Hahn 1992). The study also highlighted the less egg laying compared to the control. Magierowicz *et al*. (2019) reported elevated larval mortality alongwith reduced population percentage, emergence and longevity of *A. advenella* moths by using *T. vulgare* extracts.

In addition, steam distillate of fresh leaves and flowers of tansy, *T. vulgare* were found to be strong repellent against Colorado potato beetles. GC/MS analysis revealed the bornyl acetate (74%) as a major component (Schearer, 2004). The extracts of *T. vulgare* also exhibited the strong oviposition deterrence effect on *Tuta absoluta* Meyrick (Lep.: Gelechiidae) (Erdogan, 2019).

The infusions from dried leaves or florets of *T. patula* are popular for being used as medicinal purposes. Results of our study showed that the extracts of *T. patula* had repellent effect on Codling moth. There are only few studies which show the effect of *T. patula* extract on insects (Dutta 2007). Fabrick (2020) revealed that *T. patula* extract reduced the survival rates of *Lygus hesperus* Knight (Hemiptera: Miridae) and *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). Furthermore, Conboy *et al.* (2019) reported *T. patula* as a repellent companion plant for greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) on tomatoes in greenhouses. However, studies by Erdogan (2017) reported

that *T. patula* plants were attractive to *Tetranychus urticae* Koch (Acari: Tetranychidae), and this plant can be used as a trap plant to control *T. urticae*.

Aloe vera is widely grown as an ornamental plant and chemical analysis revealed variety of different metabolites (Eshun and He, 2004). Our study showed that the extract of *A. vera* had the highest oviposition deterrent effect on Codling moth. There is no research on the effects of *A. vera* extracts on Codling moth. However, there are many studies on the effects of *A. vera* plant extract on other insect species like aphid. Another study also reported that extract of *A. vera* had effects on larva of *Musca domestica* L. (Diptera:Muscidae) (Sarwar, 2013). Hu *et al*. (2005) reported that *A. vera* extracts exhibited strong antioxidant activities. Mallavadhani *et al.* (2016) reported that extract aloin (0.02% w/v) in *A. vera* expressed the strong repellent activity on *S. oryzae* after 24-h of exposure. Whereas, variable impact of *A. vera* extract on larvicidal activity against various instars larvae of *A. aegypti* has also been reported. The combined treatment of *A. vera* + *B. sphaericus* (1:2) material exhibited the strong larvicidal activity (Subramaniam *et al*., 2013). In addition, Zhang *et al.* (2013) reported the strong acaricidal and repellent effect of *A. vera* extracts on *Tetranychus cinnabarinus* Boisduval (Acarina:Tetranychidae). It is also revealed that the extract of *A. vera* causes the highest mortality rate of *Sitotrage cerealella* L. Lep.:Gelechiidae). Morover, Omotosa (2005) working with *A. vera* extract revealed that *A. vera* root ash showed high mortality on *Tribolium castanum* Herbst. (Coleoptera: Tenebrionidae).

Conclusion**:** *T. vulgare, H. niger* and *A. vera* plant extracts had deterrence effect on Codling moth. This is the first ever study on the oviposition deterrence effects of extracts of *T. vulgare. A. vera, H. niger, T. patula* on Codling moth. Further study is necessary for the identification of bioactive components of these plant extracts which exhibit insecticidal activities under both controlled or field conditions.

Author Contribution Statement**:** *Erdogan* planned and wrote the manuscript and *Ayse Ozdem* edited the manuscript. All the authors carefully read the paper.

Funding**:** This study was not funded by any national or international agency.

Funding**:** None

Conflicts of Interest**:** The authors declare no conflict of interest.

Acknowledgement: I would like to express my great appreciation to Ayse Ozdem for his valuable and constructive suggestion during the planning and development of this research work. His willingness to give his time so much generously has been very much appreciated.

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